

## CLAIMS

What is claimed is:

1. A method of kinase inhibition profiling, comprising:  
  
simultaneously generating a first data set having a first plurality of data elements, and  
5 a second data set having a second plurality of data elements;  
  
wherein the first data set is associated with kinase inhibition in a first kinase signaling  
pathway, wherein the second data set is associated with kinase inhibition in a  
second kinase signaling pathway, and wherein each data element in the first  
and second data sets corresponds to an inhibition result of a kinase in the first  
10 and second kinase signaling pathways, respectively; and  
  
using at least one of the data elements from the first data set as reference data against  
at least one of the data elements from the second data set to calculate a  
normalized inhibition profile.
2. The method of claim 1 wherein the step of using at least one of the data elements  
15 comprises using each of the data elements from the first data set as reference data  
against each of the data elements from the second data set to calculate the normalized  
inhibition profile.
3. The method of claim 2 wherein the inhibition result is acquired *in vivo* from a cell that  
expresses, from a stably transfected gene, and in response to an inducer, the kinase in  
20 a catalytically active form.
4. The method of claim 3 wherein inhibition of the kinase alters a signal effected by a  
recombinant reporter gene.
5. The method of claim 4 wherein the recombinant reporter gene is functionally  
controlled by a transcription factor that is activated by a component of at least one of  
25 the first and second kinase signaling pathways.

6. A method of analyzing intra-pathway kinase inhibition of a pharmaceutically active compound in a kinase signaling pathway, comprising:

providing a plurality of cells that express from a stably transfected gene and in response to an inducer a plurality of kinases in a catalytically active form,  
5 respectively, wherein the plurality of cells further express a reporter gene in response to catalytic activity of the kinases, and wherein the reporter gene in each of the plurality of cells is the same;

wherein the plurality of cells are derived from a single cell type, wherein a first kinase of the plurality of kinases is different from a second kinase of the plurality of  
10 kinases, and wherein the first and second kinases are members of the same kinase signaling pathway;

simultaneously inducing the plurality of cells with the inducer, and simultaneously presenting the cells with the pharmaceutically active compound;

acquiring from a cell expressing the first kinase a first signal effected by the reporter  
15 gene and acquiring from a cell expressing the second kinase a second signal effected by the reporter gene; and

identifying a kinase within the kinase signaling pathway as being inhibited by the pharmaceutically active compound using the first and second signals, wherein the first signal is normalized using the second signal.

- 20 7. The method of claim 6 wherein the catalytically active form is a constitutively active kinase mutant or an autophosphorylating kinase.

8. The method of claim 7 wherein the reporter gene is expressed in response to activation of a transcription factor, and wherein the activation is effected by a kinase within the kinase signaling pathway.

- 25 9. The method of claim 8 wherein the kinase signaling pathway is selected from the group consisting of a MEK-ERK mediated pathway, a IKK-NFkB mediated pathway, a p38 mediated pathway, a JNK-Jun mediated pathway, a PI3K-Akt mediated pathway, and a JAK-STAT mediated pathway.

10. The method of claim 9 wherein the inducer is doxycycline and the reporter gene is a gene encoding luciferase.
11. The method of claim 10 wherein the cells are mammalian cells.
12. The method of claim 11 wherein the mammalian cells are 293 cells.
- 5 13. A method of analyzing inter-pathway inhibition of a pharmaceutically active compound in a first kinase signaling pathway and a second kinase signaling pathway, comprising:
- providing a plurality of cells that express from a stably transfected gene and in response to an inducer a plurality of kinases in a catalytically active form, respectively, wherein the plurality of cells further express a reporter gene in response to catalytic activity of the kinases, and wherein the reporter gene in each of the plurality of cells is the same;
- 10 wherein the plurality of cells are derived from a single cell type, wherein a first kinase of the plurality of kinases is a member of a first kinase signaling pathway, and wherein a second kinase of the plurality of kinases is a member of a second kinase signaling pathway;
- 15 simultaneously inducing the plurality of cells with the inducer, and simultaneously presenting the cells with the pharmaceutically active compound;
- acquiring from a cell expressing the first kinase a first signal effected by the reporter gene and acquiring from a cell expressing the second kinase a second signal effected by the reporter gene; and
- 20 identifying a kinase within the first and second kinase signaling pathways as being inhibited by the pharmaceutically active compound using the first and second signals, wherein the first signal is normalized using the second signal.
- 25 14. The method of claim 13 wherein the catalytically active form is a constitutively active kinase mutant or an autophosphorylating kinase.

15. The method of claim 14 wherein the reporter gene is expressed in response to activation of a transcription factor, and wherein the activation is effected by an element within at least one of the first and second kinase signaling pathways.
- 5 16. The method of claim 15 wherein the first and second kinase signaling pathways are selected from the group consisting of a MEK-ERK mediated pathway, a IKK-NFkB mediated pathway, a p38 mediated pathway, a JNK-Jun mediated pathway, a PI3K-Akt mediated pathway, and a JAK-STAT mediated pathway, and wherein the first and second kinase signaling pathways are not the same kinase signaling pathway.
- 10 17. The method of claim 16 wherein the inducer is doxycycline and the reporter gene is a gene encoding luciferase.
18. The method of claim 17 wherein the cells are mammalian cells.
19. The method of claim 18 wherein the mammalian cells are 293 cells.
20. A high-throughput screening system, comprising:
- 15 a plurality of cells that express from a stably transfected gene and in response to an inducer a plurality of kinases in a catalytically active form, respectively, and wherein the plurality of cells further express a reporter gene in response to catalytic activity of the kinases;
- 20 wherein the plurality of cells are derived from a single cell type, and wherein cells expressing a first kinase of the plurality of kinases are separate from cells expressing a second kinase of the plurality of kinases;
- an acquisition system acquiring a first signal from the cells expressing the first kinase and a second signal from the cells expressing the second kinase, wherein first and second signals are effected by the reporter gene; and
- 25 a data processor that identifies a kinase as being inhibited by a pharmaceutically active compound using the first and second signals.
21. The screening system of claim 20 wherein the catalytically active form is a constitutively active kinase mutant or an autophosphorylating kinase.

22. The screening system of claim 21 wherein the reporter gene is expressed in response to activation of a transcription factor, and wherein the activation is effected by an element within a kinase signaling pathway.
23. The screening system of claim 22 wherein the kinase signaling pathway is selected from the group consisting of a MEK-ERK mediated pathway, a IKK-NFkB mediated pathway, a p38 mediated pathway, a JNK-Jun mediated pathway, and a JAK-STAT mediated pathway, and wherein the first and second kinase signaling pathways are not the same kinase signaling pathway.
24. The screening system of claim 23 wherein the reporter gene is a gene encoding luciferase and the acquisition system comprises a luminometer.
25. The screening system of claim 20 wherein the first kinase of the plurality of kinases belongs to a kinase signaling pathway that is different from a kinase signaling pathway to which the second kinase of the plurality of kinases belongs.
26. The screening system of claim 20 wherein the first and second kinases of the plurality of kinases belongs to the same kinase signaling pathway.
27. A method of data processing, comprising:
- providing a plurality of cells that express from a stably transfected gene and in response to an inducer a plurality of kinases in a catalytically active form, respectively, and wherein the plurality of cells further express a reporter gene in response to catalytic activity of the kinases;
- wherein the plurality of cells are derived from a single cell type, and wherein cells expressing a first kinase of the plurality of kinases are separate from cells expressing a second kinase of the plurality of kinases;
- acquiring with an acquisition system a first set of data elements from the cells expressing the first kinase and a second set of data elements from the cells expressing the second kinase, wherein first and second sets of data elements are measurements of signals generated by the reporter gene;

calculating a frequency of false positives and false negatives against a plurality of  
compounds to thereby validate first and second sets of data; and

calculating inhibition data from the first and second set of data elements.

28. The method of claim 27 wherein the first set of data elements of the first kinase is  
5 analyzed against the second set of data elements of the second kinase of the same or  
different kinase signaling pathway.
29. The method of claim 28 wherein the first and second kinase signaling pathways are  
selected from the group consisting of a MEK-ERK mediated pathway, a IKK-NFkB  
mediated pathway, a p38 mediated pathway, a JNK-Jun mediated pathway, and a  
10 JAK-STAT mediated pathway, and wherein the first and second kinase signaling  
pathways are same kinase signaling pathway.
30. The method of claim 28 wherein the first and second kinase signaling pathways are  
selected from the group consisting of a MEK-ERK mediated pathway, a IKK-NFkB  
mediated pathway, a p38 mediated pathway, a JNK-Jun mediated pathway, a PI3K-  
15 Akt mediated pathway, and a JAK-STAT mediated pathway, and wherein first and  
second kinase signaling pathways are not the same kinase signaling pathway.
31. The method of any one of claims 6, 13, or 20 wherein the first signal and the second  
signal comprises a radiologically detectable signal.